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Structural context for protein N-glycosylation in bacteria: The structure of PEB3, an adhesin from Campylobacter jejuni.

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Campylobacter jejuni is unusual among bacteria in possessing a eukaryotic-like system for N-linked protein glycosylation at Asn residues in sequons of the type Asp/Glu-Xaa-Asn-Xaa-Ser/Thr. However, little is known about the structural context of the glycosylated sequons, limiting the design of novel recombinant glycoproteins. To obtain more information on sequon structure, we have determined the crystal structure of the PEB3 (Cj0289c) dimer. PEB3 has the class II periplasmic-binding protein fold, with each monomer having two domains with a ligand-binding site containing citrate located between them, and overall resembles molybdate- and sulfate-binding proteins. The sequon around Asn90 is located within a surface-exposed loop joining two structural elements. The three key residues are well exposed on the surface; hence, they may be accessible to the PglB oligosaccharyltransferase in the folded state.

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Multiple N-acetyl neuraminic acid synthetase (neuB) genes in Campylobacter jejuni: identification and characterization of the gene involved in sialylation of lipo-oligosaccharide.

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N-acetyl neuraminic acid (NANA) is a common constituent of Campylobacter jejuni lipo-oligosaccharide (LOS). Such structures often mimic human gangliosides and are thought to be involved in the triggering of Guillain-Barre syndrome (GBS) and Miller-Fisher syndrome (MFS) following C. jejuni infection. Analysis of the C. jejuni NCTC 11168 genome sequence identified three putative NANA synthetase genes termed neuB1, neuB2 and neuB3. The NANA synthetase activity of all three C. jejuni neuB gene products was confirmed by complementation experiments in an Escherichia coli neuB-deficient strain. Isogenic mutants were created in all three neuB genes, and for one such mutant (neuB1) LOS was shown to have increased mobility. C. jejuni NCTC 11168 wild-type LOS bound cholera toxin, indicating the presence of NANA in a LOS structure mimicking the ganglioside GM1. This property was lost in the neuB1 mutant. Gas chromatography-mass spectrometry and fast atom bombardment-mass spectrometry analysis of LOS from wild-type and the neuB1 mutant strain demonstrated the lack of NANA in the latter. Expression of the neuB1 gene in E. coli confirmed that NeuB1 was capable of in vitro NANA biosynthesis through condensation of N-acetyl-D-mannosamine and phosphoenolpyruvate. Southern analysis demonstrated that the neuB1 gene was confined to strains of C. jejuni with LOS containing a single NANA residue. Mutagenesis of neuB2 and neuB3 did not affect LOS, but neuB3 mutants were aflagellate and non-motile. No phenotype was evident for neuB2 mutants in strain NCTC 11168, but for strain G1 the flagellin protein from the neuB2 mutant showed an apparent reduction in molecular size relative to the wild type. Thus, the neuB genes of C. jejuni appear to be involved in the biosynthesis of at least two distinct surface structures: LOS and flagella.

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